Assessment of acute mild hypoxia on retinal oxygen saturation using snapshot retinal oximetry

Assessment of Acute Mild Hypoxia on Retinal Oxygen Saturation Using Snapshot Retinal Oximetry

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Purpose. To study the effect of acute mild hypoxia on retinal oxygen saturation.

Methods. Spectral retinal images were acquired under normoxic and hypoxic conditions for 10 healthy human volunteers (six male, four female, aged 25 ± 5 years [mean ± SD]) using a modified fundus camera fitted with an image-replicating imaging spectrometer (IRIS). Acute, mild hypoxia was induced by changing the oxygen saturation of inhaled air from 21% to 15% using a hypoxia generator with subjects breathing the hypoxic gas mixture for 10 minutes. Peripheral arterial oxygen saturation of the subjects was monitored using fingertip-pulse oximetry. Images were processed to calculate oxygen saturation, arteriovenous difference in oxygen saturation, and vessel diameter. Data are presented as mean ± SD and were analyzed using paired sample *t*-test with significance accepted at $P < 0.05$.

Results. The retinal arterial and venous oxygen saturation was 98.5% ± 1.6% and 70.7% ± 2.7% during normoxia. A reduction in the fraction of inspired oxygen resulted in a decline ($P < 0.001$) in both retinal-arterial and venous oxygen saturation to 90.3% ± 2.0% and 62.4% ± 2.2%, respectively. The arteriovenous oxygen saturation difference in normoxia (27.8% ± 2.9%) and hypoxia (27.9% ± 2.1%) did not change. Retinal arteriolar and venular diameters increased ($P < 0.001$) by 4% and 3%, respectively, under hypoxia.

Conclusions. The acute inhalation of a hypoxic gas mixture resulted in a decline in both retinal-arterial and venous saturation, while arteriovenous oxygen difference was maintained with an accompanying significant increase in retinal vessel diameter. This may suggest an autoregulatory response to acute mild hypoxia.

Keywords: autoregulation, hypoxia, retina, spectral imaging, oximetry, hemoglobin

Retinal tissue has high oxygen demand and an adequate oxygen supply is required for normal function. The retina can respond to physiological variations in oxygen saturation; for example, reduced oxygen saturation can rapidly affect retinal metabolism, triggering local vasodilatation and increased blood flow.[1] Abnormal retinal tissue oxygenation has been shown to contribute to retinal diseases such as proliferative diabetic retinopathy and retinopathy of prematurity.[2,3] Furthermore, tissue oxygen consumption is partly reflected by the arteriovenous difference in oxygen saturation and may provide an objective measure of disease severity in inner retinal disease, such as glaucoma.[4] To further study retinal oxygen levels and their impact on developing retinopathy and other diseases, a reliable and noninvasive oximetry method would represent a significant development.

Retinal oximetry employing hyperspectral imaging is based on the principle that light absorption by the blood depends on both the blood-oxygen saturation and wavelength, λ, of light. In blood, hemoglobin (Hb) is one of the strongest absorbers of light and exists in varying degrees of oxygenation between the extremes of fully oxygenated (HbO_2) and fully deoxygenated (Hb) states. The absorption spectrum of partially oxygenated hemoglobin varies in proportion to the relative fraction of these two components. Oxygen saturation of blood in human retinal vessels has been determined using measurements of attenuation of light at two or more optical wavelengths.[5–8]

The retina is supplied by two vascular systems: retinal blood vessels and the choroid.[9] Retinal and choroidal blood are both derived from the ophthalmic artery, which in turn is a branch of the internal carotid artery. Whereas autoregulatory processes exist in retinal blood vessels, there is a limited degree of autoregulation in choroidal blood vessels.[10–12] Autoregulation of blood flow, through metabolic regulation, can be defined as the capacity of an organ or tissue to regulate its blood supply according to its metabolic need.[12–14] Whereas the retinal circulation is sensitive to changes in blood oxygenation, the choroid appears to be unresponsive to variations in oxygen availability.[15,16] Retinal autoregulation of blood flow is the result of the interaction of myogenic and metabolic mechanisms via the release of vasoactive substances and adaptation of the vascular tone of arterioles and capillaries.[14] Hypoxia induces vasodilation of retinal vessels, which increases retinal blood flow,[17–23] a response that can also be observed in the cerebral circulation.[24,25]

In this study, we examined the effect of acute mild hypoxia on retinal vessel oxygenation in healthy human subjects by

using retinal oximetry, arteriovenous oxygen difference, and vessel diameter.

Methods

The study was approved by the Heriot-Watt University Ethics Committee. All volunteers provided written informed consent before participation in the study. All procedures were performed in accordance with the tenets of the Declaration of Helsinki.

Ten healthy volunteers were recruited for the study (age 25 ± 5 years; six males and four females). Multispectral retinal images were acquired under both normoxia (21% fraction of inspired oxygen [FiO_2]) and hypoxia (15% FiO_2) conditions using a modified commercial fundus camera (Topcon TRC 50 IA; Topcon, Itabashi, Tokyo, Japan) fitted with an image-replicating imaging spectrometer (IRIS), which acquires images in a single snapshot at eight different wavelengths optimized for oximetry.[26–29] Hypoxia was induced by a hypoxia generator (Everest Summit II Hypoxic Generator; Hypoxico, Inc., New York, NY) by reducing the FiO_2 from 21% to 15%. The hypoxia generator was calibrated prior to use, using a commercial gas analyzer (Servomex Company, Inc., Sugar Land, TX) that measured the percentage of oxygen output of the system to ±0.1%. The performance of the hypoxia generator is described elsewhere.[30] Fingertip pulse oximetry (AUTOCORR; Smiths Medical ASD, Inc., Rockland, MA) was employed to continuously monitor the peripheral arterial oxygenation throughout the experiment. Pupils were dilated before retinal imaging with 1% tropicamide (Bausch & Lomb, Chauvin Pharmaceuticals, Ltd., Kingston-upon-Thames, Surrey, UK).

Algorithms exploiting optical absorption measurements in all eight spectral bands are under development and offer the future prospect of robust, calibration-free oximetry. This work will be reported in future publications. The retinal oximetry used in this study is based on the calibration-based, two-wavelength oximetry by Beach et al.,[5] which is a reputable reference technique. This technique involves calibration of optical density ratios (ODRs) of arteries and veins assuming accepted blood oxygenation obtained from oxygen saturation measurements in healthy volunteers.[31] Of the eight images captured in a single snapshot (see Fig. 1), the two recorded at 566 nm and 599 nm (spectral full width of 7 nm) were selected for oximetry since we have determined, using modeling and experimental assessment, that they provide the lowest variation in oximetry along single vessels. The first of these is optimized to be insensitive to blood oxygenation while the second shows a near-optimal variation of absorption with oxygenation for blood vessels with a caliber of approximately 100 μm. The center wavelength of the 566-nm band is slightly displaced from the

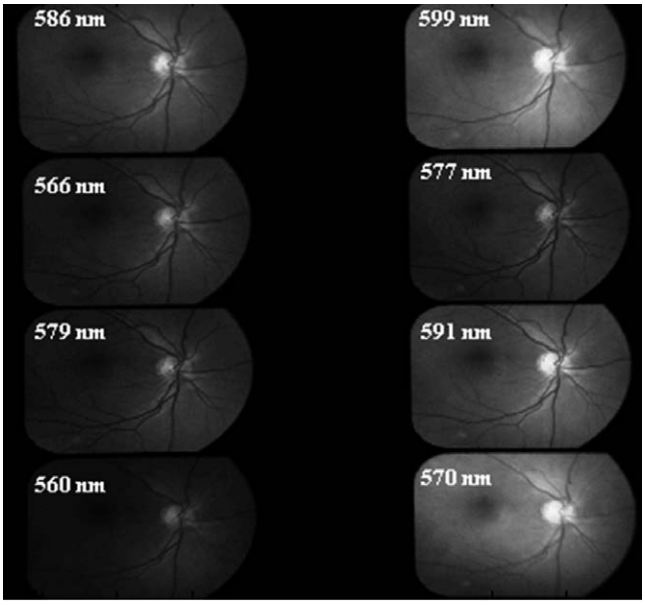

Figure 1. Raw image captured by IRIS snapshot device, fitted with fundus camera, each image contains eight subimages at different wavelengths.

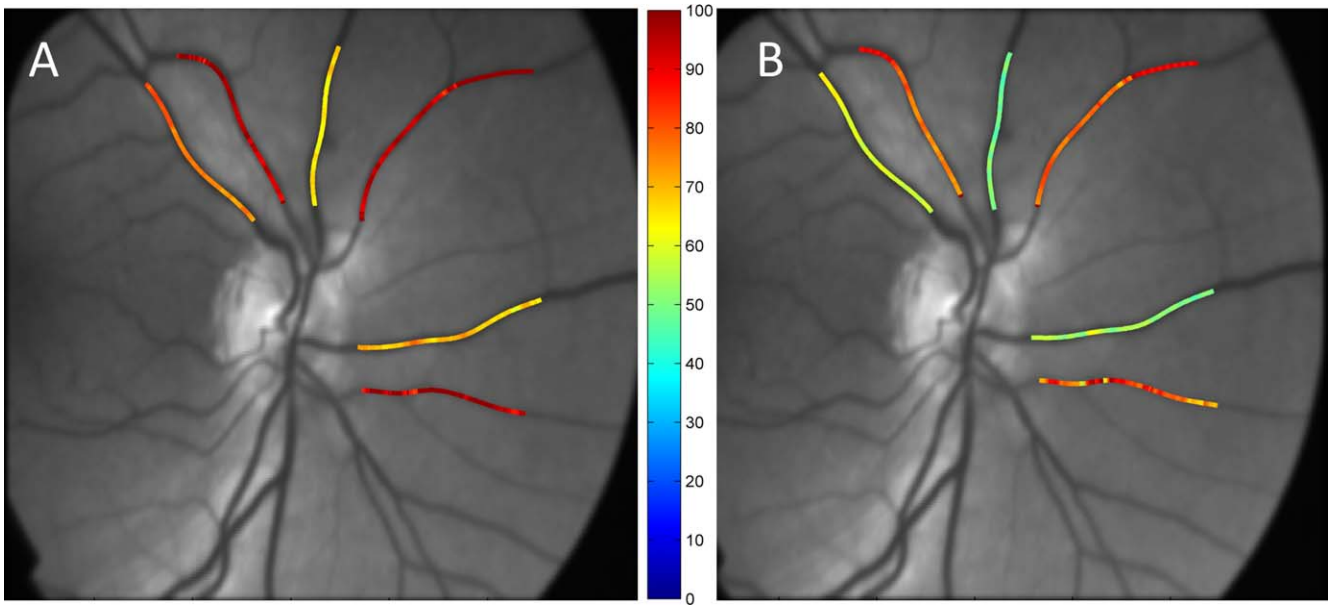

Figure 2. Oximetry image of the retina, where oximetry is shown for every pixel along each blood vessel, prior to aggregation. Images are of the same eye: (**A**) is normoxic and (**B**) is hypoxic. The same vessel sections were selected in both images to compare oxygen saturation and vessel diameter.

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Table 1. Oximetry and Vessel Diameter Values of Five Images Within the Same Subject

	Arterioles		Venules	
Subject No.	O_2 Saturation, mean ± SD, %	Arteriole Diameter, mean ± SD, pixels	O_2 Saturation, mean ± SD, %	Venule Diameter, mean ± SD, pixels
1	98.6 ± 1.0	14.4 ± 0.3	71.3 ± 0.7	18.5 ± 0.4
2	98.3 ± 0.7	15.2 ± 0.3	72.2 ± 0.4	18.5 ± 0.3
3	97.0 ± 0.5	13.6 ± 0.3	69.2 ± 1.0	18.9 ± 0.2
4	99.4 ± 0.4	14.6 ± 0.2	71.9 ± 0.5	18.4 ± 0.4
5	98.1 ± 0.5	14.5 ± 0.3	65.7 ± 0.6	18.9 ± 0.2
CV, %	1	4.1	3.5	2.1

In each subject, one arteriole and one venule were measured to assess repeatability of measurements. CV = (SD/Mean × 100).

monochromatic isosbestic wavelength of 569 nm due to the finite width and a slight asymmetry of the spectral pass-band of the filter. Orthogonal linear polarization of illumination and imaging channels effectively eliminates the specular reflex from the center of the vessels.

Optical density (OD) was calculated for the larger vessels at these two wavelengths. The OD is the ratio of the measured light intensity at the center of the vessel to the intensity just outside of a vessel:

$$OD = -\log_{10}\left(\frac{I_V}{I_R}\right) \quad (1)$$

where I_V and I_R are the intensities of light reflected from the vessel and adjacent to the vessel, respectively. That is, OD (also known as absorbance) represents the absorption of light by the blood vessel. The ODR at two wavelengths ($ODR = OD_{599}/OD_{566}$) has an inverse and linear relationship to oxygen saturation.[5]

Retinal images under normoxia and hypoxia were recorded for each subject and processed to track vessels, calculate *OD* and *ODR,* and hence calculate oxygen saturation at each pixel along the centerline of the selected vessels. For each subject, arterial and venous oxygen saturation was then calculated for each point along a vessel for each level of inspired oxygen. Vessel segments were selected in a standardized manner, based on vessel width (12 pixels or wider) and vessel length (100–200 pixels). Furthermore, parts of the vessels close to the optic disk were avoided. Care was also taken to exclude vessels with strong background variations in reflectivities that are known to exhibit higher levels of artifactual errors in oximetry. Oximetry was averaged along major vessel segments (between branches) yielding a measure of mean and standard deviation of the oximetry by vessel segment for each image. The same arterioles and venules were selected under normoxic and hypoxic conditions and comparison of oximetry between normoxic and hypoxic conditions was performed between the same vessel segments. An example oximetry is shown in Figure 2. While one would expect an approximately constant blood oxygenation along a vessel, the false-color scheme highlights the small, artifactual, systematic, and random variations in oximetry. Random variations arise from image–noise-related effects while larger systematic variations correlate strongly with background reflectivity and between the normoxic and hypoxic oximetries of the same vessel. The comparison of data at the scale of a vessel segment provides good averaging of both systematic and random variations in oximetry, while enabling the identification of intraretinal variations in oximetry.

The full-width at half maximum of the retinal vessel diameters of the vessels selected for oximetry were measured under normoxic and hypoxic conditions using algorithms based on the method reported by Fischer et al.[32] Similar to the method used to aggregate and compare oximetry data, vessel diameters were averaged by vessel segment and compared on a segment-by-segment basis between normoxic and hypoxic eyes. As the magnification of the system—including the eye focal length—is not accurately known, we present the vessel diameters in terms of pixels; an intersubject accurate comparison of diameters is not possible.

To assess the significance of any hypoxia-induced intrasubject variations in oximetry and vessel diameter, it is important to assess whether the variation between nominally identical measurements is sufficiently small compared with the magnitude of the observed changes with hypoxia. This was assessed using five repeated measurements on five subjects using identical procedures to those used throughout this study. The retinal images were recorded at 1-minute intervals, with the camera (Topcon TRC 50 IA; Topcon) realigned and refocused as necessary. Vessel oximetries and vessel diameters were calculated for one arteriole and one venule in each eye for each of the five individuals. Results from the repeatability experiment are included in the Results section. Data were analyzed using a paired-sample *t*-test with significance accepted at $P < 0.05$, and are presented as mean ± SD.

Table 2. Oxygen Saturation and Vessel Diameter Values for Subjects Under Normoxia and Hypoxia Conditions

Demographics and O_2 Saturation Values for Subjects	Normoxia	Hypoxia	*P**
Retinal arteriole O_2 saturation, mean ± SD, %	98.5 ± 1.6	90.3 ± 2.0	<0.001
Retinal venule O_2 saturation, mean ± SD, %	70.7 ± 2.7	62.4 ± 2.2	<0.001
Retinal arteriovenous saturation, mean ± SD, %	27.8 ± 2.9	27.9 ± 2.1	0.31
Fingertip pulse O_2 saturation, mean ± SD, %	98.6 ± 0.7	89.6 ± 0.5	<0.002
Vessel diameter arteriole, pixels	14.4 ± 1.2	15.0 ± 1.1	<0.001
Vessel diameter venule, pixels	18.7 ± 1.0	19.3 ± 1.1	<0.001
Age, mean (total range), y	25 (22–51)	25 (22–51)	—
Sex distribution	6/4	6/4	—

* Paired *t*-test.

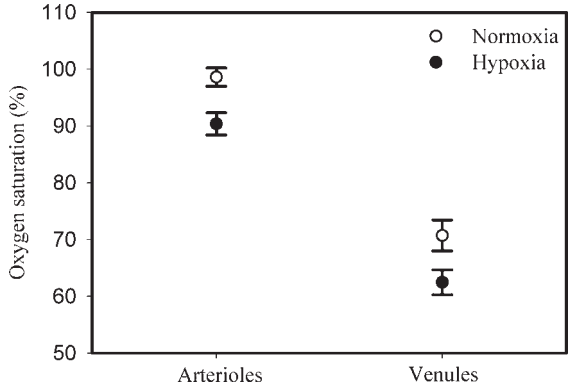

FIGURE 3. The effect of hypoxic exposure on oxygen saturation in normoxia (*open circle*) and hypoxia (*closed circle*). The *open* and *closed circles* represent the mean while the *errors bars* indicate the standard deviations.

RESULTS

Repeatability of Oximetry System and Vessel Diameter Calculation

Table 1 shows the oxygen saturation and vessel diameter as the mean ± SD of repeated measurements in five subjects. The intrasubject standard deviation for five repeated oximetry calculations for arterioles and venules of five subjects varies between 0.4% and 1.0%. The intrasubject standard deviation for repeated vessel diameter calculation was between 0.2 and 0.3 pixels for arterioles and 0.3 and 0.4 pixels for venules.

Normoxia

Under normoxia, the pulse oximetry was found to be 98.6% ± 0.7%; the retinal arterial O_2 saturation closely matched this value (98.5% ± 1.6%) and the retinal venous O_2 saturation was 70.7% ± 2.7%. The arteriovenous difference was calculated to be 27.8% ± 2.9% (mean ± SD) under normoxic conditions (see Table 2 for values). A comparison of oxygen saturation in the arterioles and venules in subjects at normoxia is shown in Figure 3.

Comparison of Oxygen Saturation in Normoxia and Hypoxia

A reduction in the fraction of inspired oxygen resulted in a decline ($P < 0.001$) in the pulse oximetry value to 89.6% ± 0.5%. The retinal arterial and venous oxygen saturations reflected this decrease and were found to be 90.3% ± 2.0% and 62.4% ± 2.2%, respectively, with an arteriovenous difference of (27.9% ± 2.1%). A comparison of oxygen saturation in the arterioles and venules in subjects at normoxia and hypoxia is shown in Figure 3.

Retinal Vessel Diameter in Normoxia and Hypoxia

Figure 4 shows the effect of hypoxic exposure on retinal vessel diameter. Retinal vessel (arterioles and venules) diameter (in pixels) was measured from the retinal images of subjects under both normoxic and hypoxic conditions. During normoxia, retinal arteriole and venule diameter was 14.4 ± 1.2 and 18.7 ± 1.0 pixels, respectively. There was a small but significant increase ($P < 0.001$, paired *t*-test) in retinal vessel diameter under hypoxic conditions (arteriole, 15.0 ± 1.1; venule, 19.3 ± 1.1). The paired *t*-test is between the same vessel sections in the same eyes.

DISCUSSION

In this study, the effect of acute mild hypoxia on retinal oxygenation was examined. We can draw the following conclusions from the results:

1. A reduction in the fraction of inspired oxygen (from 21% to 15%) resulted in a decline ($P < 0.001$) in retinal-arterial and venous oxygen saturation.
2. Despite the decrease in arterial and venous oxygen saturation, we calculated that the arteriovenous oxygen saturation difference remained unchanged during hypoxia when compared with normoxia.
3. During acute mild hypoxic exposure, the diameters of the retinal-arterial and venous vessels were observed to increase by a small but highly significant ($P < 0.001$) increment of 4% and 3%, respectively. This increase in retinal vessel diameter may suggest an autoregulatory response to meet the metabolic demand under conditions of hypoxia.

Mild hypoxia was induced by reducing the FiO_2 to 15%. This resulted in decreased oxygen saturation in both the retinal arterioles and venules, while the arteriovenous oxygen-saturation difference remained unchanged. The pulse oximeter reading during the hypoxia phase of the experiment agreed with the observed decrease in retinal arterial O_2 saturation. We did not measure the blood flow directly, but postulate that there should be an increase in blood flow since we observed a significant increase in retinal vessel diameter. Increase in retinal vessel diameter and blood flow has also previously been reported to accompany hypoxia,[17–23] and measurement of the changes in retinal vessel diameter and arteriovenous oxygen difference have previously been employed to estimate the relative change in retinal blood flow.[33] Assuming, as stated by Poiseuille's law,[34] that blood flow increases with the fourth power of the caliber, then the 3.5% average increase in vessel diameter that we measured corresponds to an increase in blood flow of 16%. The rate of oxygen delivery is equal to the product of the arteriovenous oxygen difference and blood flow, therefore suggesting that the absolute magnitude of oxygen delivery by the retinal vessels increased during hypoxia. This increase may compensate for a reduced contribution from the choroidal circulation, which is reported to be insensitive to changes in oxygen saturation.[15,16]

This is one of the first studies to assess the effect of acute mild hypoxia (10-minute exposure) on retinal circulation. Previous studies on retinal circulation have examined the effect of severe and chronic hypoxia over a longer period of time (from hours to days). Collectively, these studies imply that hypoxia induces vasodilation of the retinal vessels and increases retinal blood flow.[17–23] In a study conducted at an altitude of 5300 m (partial pressure of oxygen is equivalent to 10% inspired oxygen at sea level), it was reported that the retinal arterial and venous diameter increased by 18% and 21%, respectively, within 2 hours of exposure to altitude.[23] In another study,[17] a moderate but significant increase in retinal vessel diameter was observed in monkeys during mild hypoxia ($PaO_2 = 59$ mm Hg, which approximates FiO_2 of 15%). This is consistent with our measurements on humans. At a severe level of hypoxia ($PaO_2 = 35$ mm Hg, approximates FiO_2 of 11%), vasodilation was more pronounced.

In many retinal diseases including diabetic retinopathy, glaucoma, and age-related macular degeneration, retinal circulation and functions are impaired. In these diseases, retinal circulation does not properly respond to hypoxia, flicker stimulation, or dark adaptation.[12,14,35] Therefore, improved understanding of the effects of hypoxic stress on

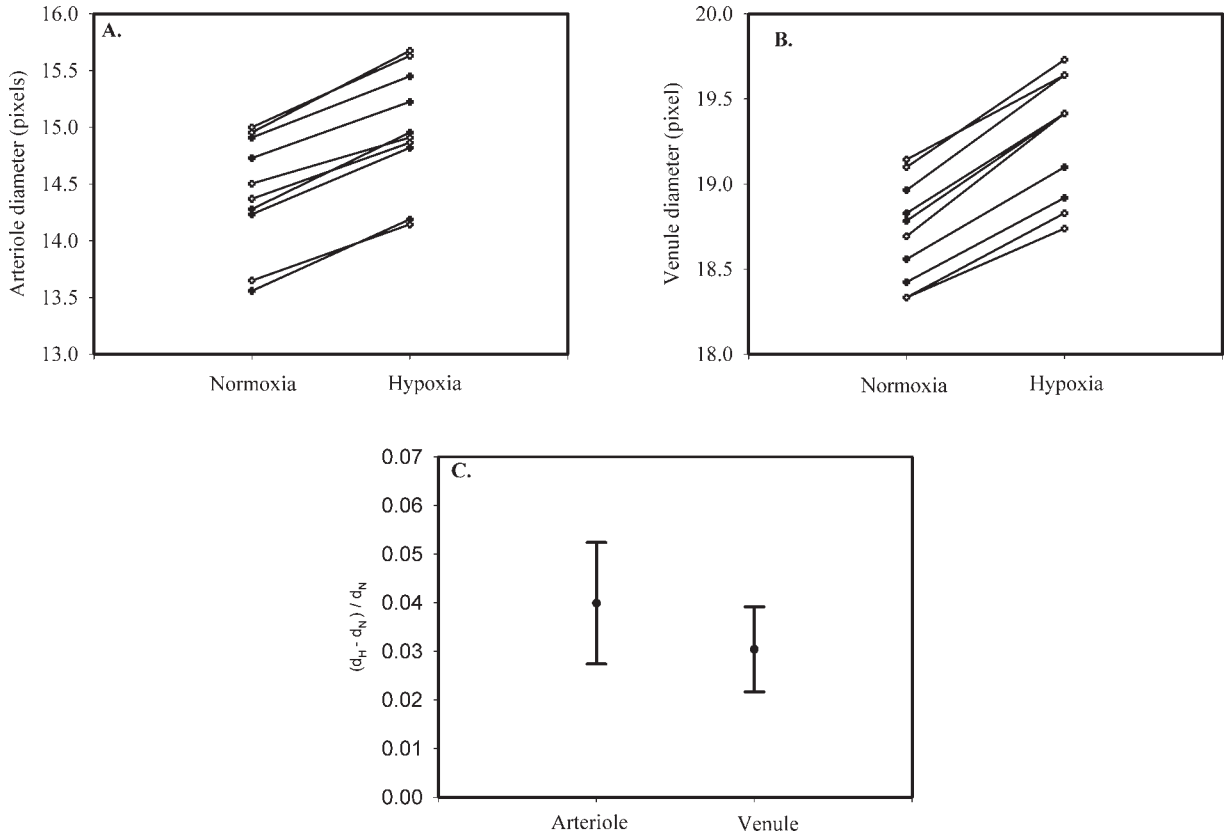

Figure 4. The effect of hypoxic exposure on vessel diameter (in pixels) in (**A**) arterioles and (**B**) venules compared with normoxia. (**C**) Fractional increase, $(d_H - d_N)/d_N$ in diameter, where d_H and d_N are vessel diameter in pixels during hypoxia and normoxia, respectively.

retinal oxygenation and functions in normal healthy individuals such as we describe here provides a route to developing an understanding of these disease mechanisms and potentially for clinical diagnosis.

One might also consider the potential to use imaging of retinal circulation as a convenient indicator of cerebral circulatory processes. However, despite the many similarities between the cerebral and retinal circulation,[36,37] it should also be recognized that differences also exist. For example, the intraocular pressure is higher than in the brain and the retinal vasculature is more sparse leading to a higher arteriovenous oxygen difference. To discuss the similarities and differences between them is neither the aim nor within the scope of this paper. We highlight here this issue as an indication of the merit for further studies to establish the value of this potential.

We describe here an application of two-wavelength oximetry using an established calibration technique that employs assumed values of oxygenation of veins and arteries in healthy eyes based on normal physiology. One of the limitations of this study is that it is based on calibration-based, two-wavelength retinal oximetry. With this technique, absolute oximetry is not possible. Our calculated venous oximetry of 70% is higher but comparable with values reported by Hammer et al.[38] However, in this study, the conclusions are based on changes in measured blood oxygenation and these conclusions are unaffected by a scaling or offset of oximetry with respect to absolute values. We report here a significant increase in vessel caliber during hypoxia, which corresponds to an increase in blood flow, although we did not measure blood flow directly to affirm this conclusion.

Conclusions

The retinal-arterial oxygen saturation recorded using a novel snapshot spectral retinal camera under normoxia significantly correlated with the pulse oximetry values ($r = 0.96$, $P < 0.0001$, Spearman's rank correlation test). The acute inhalation of a hypoxic gas mixture resulted in a decline in both retinal-arterial and venous saturation, as well as a significant increase in retinal vessel caliber, suggesting an autoregulatory response. Our study, on a small group of normal volunteers, suggests that this retinal-oximetry method using spectral imaging is reliable and sensitive to small changes in oxygen saturation and retinal vessel caliber. The ability to perform noninvasive oximetry in retinal vessels in vivo allows assessments of retinal circulation in health and disease.

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